AD_____

Award Number: W81XWH-08-1-0777

TITLE: The Effects of Bisphenol A (BPA) on ErbB2 Positive Breast Cancer

PRINCIPAL INVESTIGATOR: Sarah B. Jenkins

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
Birmingham, AL 35294-0109

REPORT DATE: November 2009

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

x Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
30-11-2009	Annual Summary	1 Nov 2008 - 31 Oct 2009

4. TITLE AND SUBTITLE

The Effects of Bisphenol A (BPA) on ErbB2-Positive Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-08-1-0777

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Sarah Jenkins

Email: jenkinss@uab.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Alabama at Birmingham
1530 3rd Avenue South, AB990
Birmingham, AL, 35294-0111

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Material Command
Fort Detrick, Maryland
21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this grant was to investigate the ability of BPA, when administered orally, to alter tumorigenesis in a transgenic mouse model that over-expresses the erbB2 proto-oncogene (erbB2-tg). BPA did not function in a traditional, linear dose-responsive manner to induce tumorigenesis. Our data indicate that the environmentally relevant concentrations of BPA (BPA 2.5 and BPA 25) function in a different manner than the regulatory-based concentrations of BPA (BPA 250 and BPA 2500). Only the environmentally relevant BPA doses significantly accelerated mammary tumorigenesis, decreasing tumor latency and increasing tumor multiplicity, burden, and the rate of metastasis. Prior to tumor formation, all BPA doses significantly increased the rate of cell proliferation in the mammary gland, but only the regulatory-based doses increased the rate of apoptosis. Because environmentally relevant concentrations of BPA failed to increase apoptosis to counter the increased rate of proliferation, the ratio of proliferation-to-apoptosis was altered. This ratio best predicted the tumorigenic response of erbB2-tg mice to each dose of BPA studied.

15. SUBJECT TERMS

Bisphenol A, ErbB2, oral administration, non-monotonic dose response

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(Include area code)*

Table of Contents

INTRODUCTION:
Bisphenol A (BPA) is a synthetically made compound used to produce a myriad of commonly used consumer goods. Studies have found that BPA is capable of leaching from these products in appreciable amounts, resulting in greater than 90% of study participants having detectable concentrations of urinary BPA. Due to BPA's endocrine disrupting effects and widespread exposure, it is suspected to play a role in breast cancer development and progression. We have previously shown that prepubertal exposure (days 2-20 postpartum) to BPA resulted in increased tumor multiplicity and decreased latency in a model of chemically induced rodent mammary cancer. Prepubertal exposure to BPA caused increased cell proliferation, decreased apoptosis, increased expression of erbB3 (the preferred binding partner of erbB2), and increased activation of the downstream Akt pathway in the mammary gland. The purpose of this grant was to investigate the ability of BPA, when administered orally, to accelerate tumorigenesis in a transgenic mouse model that over-expresses the erbB2 proto-oncogene (ErbB2-tg). Clinically, this mouse model represents an aggressive pathology of a large subset (15-30%) of breast cancer cases.

BODY:

Aim 1a: To investigate the ontogeny of mammary pathology in erbB2-tg mice in order to identify the exact time to pathology under control conditions (Months 1-3).

Task 1a2: Pathology- This task has been completed, thereby completing Aim 1a.

The erbB2-tg mouse model was characterized under control conditions and set-up for use in this laboratory. Time points for subsequent aims were selected based on the ontogeny of mammary gland disease development and indices of cell proliferation and apoptosis prior to tumor development. Sixteen weeks was selected as the end point for mechanistic studies. At this age, no preneoplastic/neoplastic lesions had been identified in the mammary glands of untreated mice. Also, untreated mice had not yet achieved the peak or trough index value for either cell proliferation or apoptosis. Thirty-six weeks of age was selected as the end point for the tumorigenesis study. At this age, there was an equal mix of well- and moderately-differentiated tumors identified in untreated mice. Further, there was no evidence of pulmonary metastasis in untreated mice at this age.

Aim 1b: To identify the dose-dependent impact of BPA on mammary cancer progression in terms of palpable tumor latency, multiplicity, and pulmonary metastasis in a model of spontaneously developing mammary cancer (Months 1-8).

Task 1b.1-4: BPA Treatment, Palpations, Dissections, and Histopathology- These tasks are complete, thereby completing Aim 1b.

BPA did not elicit a traditional, linear dose-responsive effect on carcinogenesis in erbB2-tg mice. Only the lowest dose selected for use (BPA 25) significantly accelerated tumorigenesis. The higher doses of BPA (BPA 250 and BPA 2500) failed to differ significantly from control. The positive control, estradiol (E2), also failed to significantly alter tumorigenesis. Despite its frequent existence in the literature, a non-monotonic dose response is a controversial topic. In order to support these findings of the lower, but not the higher, doses of BPA accelerating carcinogenesis, we performed another tumorigenesis study. This study functioned two-fold: 1) It provided another independent study that duplicated our previous non-monotonic dose response, and 2) it included an additional BPA dose, one that was a 10-fold reduction from our previously defined lowest dose. Table 1 shows all of the doses used, the resultant estimated intake in mice (assuming 20 grams body weight and four ml water intake daily), and the significance of each dose. These doses can be divided into two categories, environmentally relevant (BPA 2.5 and BPA 25) and regulatory-based (BPA 250 and BPA 2500) doses of BPA.

Table 1: Estimated Daily Intake of BPA for MMTV-erbB2 Transgenic Mice

Group	Treatment	Estimated Daily Intake	Significance
Control	None	0 µg BPA/kg BW	Baseline
BPA 2.5	2.5 µg BPA/L	0.5 µg BPA/kg BW	Adult Exposure (low)
BPA 25	25 µg BPA/L	5 µg BPA/kg BW	Adult Exposure (high)
BPA 250	250 µg BPA/L	50 µg BPA/kg BW	EPA reference dose
BPA 2500	2500 µg BPA/L	500 µg BPA/kg BW	1% NTP's LOAEL

Low, environmentally relevant concentrations of BPA (BPA 2.5 and BPA 25) were found to significantly decrease tumor latency while increasing tumor multiplicity, tumor burden, and the rate of pulmonary metastasis in erbB2-tg mice. These effects were not observed in the higher, regulatory-based doses of BPA (BPA 250 and BPA 2500) or in the E2 treatment group. The details of this data can be found in Table 2.

Table 2: Tumorigenesis Data at 36 Weeks of Age

Treatment	Tumor Multiplicity	Tumor Latency	Tumor Burden	Rate of Metastasis
Control	1.03 ± 0.12	239 days	25.88 ± 3.05	3 %
BPA 2.5	1.63 ± 0.25 *	210 days *	34.77 ± 5.62 *	14 % *
BPA 25	1.52 ± 0.17 *	203 days *	42.66 ± 5.82 *	13 % *
BPA 250	1.00 ± 0.16	240 days	24.04 ± 6.28	3 %
BPA 2500	0.97 ± 0.17	210 days	20.87 ± 5.58	3 %
E2	1.06 ± 0.21	208 days	23.51 ± 5.50	3 %

Female MMTV-erbB2 mice were exposed to 0 (n=95), 2.5 (n=37), 25 (n=76), 250 (n=37), and 2500 (n=36) µg BPA/L drinking water or 54 ng E2/L (n=31) drinking water from eight to 36 weeks of age.
** Represents a statistically significant p-value (p≤0.05) as compared to control.*

The higher, regulatory-based doses of BPA and E2-treated mice significantly altered body weight or uterine wet weight. BPA 250, BPA 2500, and the E2 treatment groups significantly decreased body weight, though this reduction did not reach toxicological significance (>10% loss). When corrected for body weight, uterine wet weights were significantly increased in both BPA 250 and BPA 2500, supporting existing evidence that BPA functions as a weak estrogen only at high concentrations. The details of this data can be found in Table 3.

Table 3: Body and Uterine Weights at 36 Weeks of Age

Treatment	BW (g)	Uterine Weight (mg)	UtW:BW Ratio
Control	25.98 ± 0.32	90.84 ± 4.30	3.52 ± 0.17
BPA 2.5	25.16 ± 0.59	107.24 ± 8.23	4.32 ± 0.34
BPA 25	25.88 ± 0.38	107.25 ± 5.98	4.21 ± 0.25
BPA 250	24.25 ± 0.25 *	123.32 ± 13.63	5.14 ± 0.57 *
BPA 2500	24.39 ± 0.27 *	113.04 ± 6.51 *	4.64 ± 0.26 *
E2	24.00 ± 0.35 *	104.34 ± 5.75	4.37 ± 0.24

Female MMTV-erbB2 mice were exposed to 0 (n=95), 2.5 (n=37), 25 (n=76), 250 (n=37), and 2500 (n=36) µg BPA/L drinking water or 54 ng E2/L (n=31) drinking water from eight to 36 weeks of age.
** Represents a statistically significant p-value (p≤0.05) as compared to control.*

Aim 2: To determine the mechanism of action behind BPA's effects on promoting mammary carcinogenesis in erbB2-tg over-expressing mice (Months 1-24).

Task 2.3: Histological Staining (months 1-4)- This task is complete.
Task 2.4: Apoptosis & Proliferation (months 1-2)- This task is complete.
Task 2.5: IHC Staining (months 3-6)- This task is ongoing.
Task 2.6: Western Blotting (months 5-24)- This task is ongoing.

Task 2.7: 2D Gels (months 6-20)- This task is complete. Unfortunately, all of the proteins identified to be significantly different represent high abundance proteins. Thus, while complete, this aim yielded little interpretable insight into the action of BPA. We are optimistic that western blotting a IHC will yield more valuable insight.

All doses of BPA investigated significantly increased the rate of cell proliferation in the mammary glands of erbB2-tg mice. However, only the higher, regulatory-based doses of BPA increased the rate of apoptosis in the mammary gland, with BPA 2500 achieving statistical significance. The ratio of cell-proliferation-to-apoptosis best predicted the response of the erbB2-tg model to each dose of BPA. BPA 2.5 and BPA 25 had an increased proliferation-to-apoptosis ratio, with BPA 25 being statistically significant as compared to control. The details of this data can be found in Table 4.

Table 4: Cell Turnover in the Mammary Gland

Treatment	Proliferation Index	Apoptosis Index	Ratio
Control	0.08 ± 0.01	0.007 ± 0.001	20.30 ± 4.8
BPA 2.5	0.18 ± 0.03 *	0.004 ± 0.0006	48.33 ± 11.1
BPA 25	0.33 ± 0.03 *	0.004 ± 0.001	153.05 ± 54.7 *
BPA 250	0.30 ± 0.03 *	0.015 ± 0.004	20.22 ± 6.4
BPA 2500	0.30 ± 0.06 *	0.031 ± 0.008 *	21.42 ± 10.6
E2	0.21 ± 0.04 *	0.010 ± 0.004	9.40 ± 2.11

*Female MMTV-erbB2 mice were exposed to 0 (n=15), 2.5 (n=8), 25 (n=8), 250 (n=8), and 2500 (n=8) µg BPA/L drinking water or 54 ng E2/L (n=8) drinking water from eight to 16 weeks of age. * Represents a statistically significant p-value (p≤0.05) as compared to control.*

Task 2.5 and 2.6 are ongoing. All tissues have been collected. Antibodies have been ordered and are currently being optimized.

Aim 3: To assess whether exposure to BPA in erbB2-tg mice results in the accumulation of BPA in mammary tumors or adipose tissue (Months 14-24).

Task 3.1-3: BPA Treatment, Dissections, and BPA Measurements (months 14-24)

To date, we have not been successful in the extraction and measurement of BPA metabolites from mammary tumors or adipose tissue. However, we have contacted Dr. Antonia Calafat of the Center for Disease Control for the measurement of BPA metabolites in the serum of these mice. This is an established procedure in the CDC lab. However, they have been hesitant to measure tumor or mammary gland tissue concentrations of BPA before the corresponding serum concentrations of BPA are known. In the interim, we are looking to collaborate with the UAB mass spectrometry core facility to begin working out methods to extract BPA from normal and cancerous mammary tissue. If we are unsuccessful in this, we will contact the DoD contracting officer for this grant in order to alter the Statement of Work accordingly, substituting serum concentrations of BPA for tissue concentrations.

Aim 4: To investigate the potential of BPA exposure to be correlated with altered sex steroid and growth factor mediated signaling in human clinical samples of breast tumors in women diagnosed with HER2/erbB2-positive breast cancer (Months 1-24).

Task 4.1 & 4.2: Recruitment and Sample Collection (months 1-24)- Recruitment is ongoing. To date, we have entered a total of 36 women into the study. Paired urine and breast tumor tissue from these women has been collected, aliquoted, and stored.

Task 4.3 & 4.4: Measuring BPA Concentrations and Breast Tumor Biopsies- This aim is scheduled in take place in months 22-24.

Recruitment has not gone as well as expected. To date, only six of the 36 women entered into the study represent breast cancers that are HER2-positive. This is due to most women with a HER2-

positive pathology being placed on neoadjuvant therapy prior to surgery, thus making them ineligible for participation in this study.

KEY RESEARCH ACCOMPLISHMENTS:

Aim 1a: Ontogeny Study of the ErbB2-tg Mouse Model

- Sixteen and 36 weeks of age were selected for all downstream mechanistic and tumorigenesis studies, respectively.

Aim 1b: BPA Tumorigenesis in ErbB2-tg Mice

- BPA 2.5 and BPA 25 significantly accelerated tumorigenesis by decreasing tumor latency and increasing tumor multiplicity, tumor burden, and the rate of pulmonary metastasis.
- When corrected for body weight, BPA 250 and BPA 2500 significantly increased the uterine wet weight, supporting that BPA functions as an estrogen only at high concentrations.
- BPA resulted in a dose-dependent decrease in body weight, with BPA 2500 achieving statistical, but not toxicological, significance.

Aim 2: BPA's Mechanism of Action

- All doses of BPA significantly increased the rate of cell proliferation in the mammary gland. Only the regulatory-based doses of BPA increased the rate of apoptosis in the mammary gland, with BPA 2500 achieving statistical significance. This resulted in an altered ratio of cell-proliferation in the low, environmentally relevant concentrations of BPA but not the regulatory-based concentrations of BPA.

REPORTABLE OUTCOMES:

Awards: (1) Society of Toxicology Student Travel Award, (2) Graduate Student Association Travel Grant, (3) Susan G. Komen Travel Scholarship, (4) 3rd Place in UAB's Graduate Student Research Days Presentation, and (5) William C. Baily Award for Excellence in Cancer Prevention and Control.

Abstracts:

- Jenkins S, Kennerly R, and Lamartiniere CA. Bisphenol A & the Non-Monotonic Dose Response: Why Environmentally Relevant Doses Pose More Danger than Pharmacological Doses. PPTOXII (Miami Beach, FL). December 2009.
- Jenkins S, Kennerly R, and Lamartiniere CA. Bisphenol A & the Non-Monotonic Dose Response: Why Environmentally Relevant Doses Pose More Danger than Pharmacological Doses. UAB Comprehensive Cancer Center Research Retreat (Birmingham, AL). October 2009.
- Jenkins S and Lamartiniere CA. Sustained Exposure to Low Doses of BPA Results in a Non-Monotonic Dose Response Curve of Tumorigenesis in the MMTV-erbB2 Transgenic Mouse Model. Gordon Conference: Hormone Action in Development & Cancer (Holderness, NH) July 2009.
- Jenkins S, Betancourt A, Mobley J, Lamartiniere CA. Chronic Bisphenol A Exposure Accelerates Mammary Tumorigenesis and Alters the Mammary Proteome. 3rd European Proteomics Association Congress (Stockholm, Sweden) June 2009.
- Jenkins S, Lamartiniere CA. Chronic Bisphenol A Exposure during Adulthood Accelerates Mammary Carcinogenesis. Breast Cancer and the Environment Research Centers' Fifth Annual Early Environmental Exposure Meeting (Birmingham, AL), November 2008.
- Jenkins S, Raghuraman N, Lamartiniere CA. Early Exposure to Bisphenol A Imparts Increased Tumorigenesis and Long-Lasting Alterations to the Protein Expression in the Mammary Glands of Adult Rats. Breast Cancer and the Environment Research Centers' Fifth Annual Early Environmental Exposure Meeting (Birmingham, AL), November 2008.
- Jenkins S, Lamartiniere CA. Evaluating the Impact of Adult Exposure to Bisphenol A on Women with Breast Cancer. Breast Cancer and the Environment Research Centers' Fifth Annual Early Environmental Exposure Meeting (Birmingham, AL), November 2008.

- Jenkins S, Lamartiniere CA. Adult Exposure to the Plastic Component, Bisphenol A, Accelerates ErbB2-Positive Mammary Cancer in Mice. UAB Comprehensive Cancer Center Research Retreat (Birmingham, AL). November 2008.

Oral Presentations:

- Jenkins S and Lamartiniere CA (2009) *Chronic Exposure to the Plastic Component, Bisphenol A, Accelerates Mammary Carcinogenesis.* February 2009. Department of Pharmacology & Toxicology seminar series, UAB.
- Jenkins S and Lamartiniere CA (2009) *Chronic Exposure to the Plastic Component, Bisphenol A, Accelerates ErbB2-Positive Mammary Cancer*. February 2009. Graduate Student Research Days, UAB.

Manuscripts:

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, and Lamartiniere CA (2009) Oral Exposure to Bisphenol A Increases Dimethylbenzanthracene-Induced Mammary Cancer in Rats. *Environmental Health Perspectives*. 117(6):910-5

CONCLUSIONS:

When BPA is administered orally to erbB2-tg mice, it does not function in a traditional, linear dose-responsive manner to induce tumorigenesis. Instead, it was only the lower doses of BPA studied (BPA 2.5 and BPA 25), those doses which are achievable through normal dietary intake, that are capable of significantly accelerating mammary tumorigenesis in this model. The higher, regulatory-based doses of BPA (BPA 250 and BPA 2500) and the E2 positive control failed to significantly alter tumorigenesis. However, these treatment groups (BPA 250, BPA 2500, and E2) did significantly alter overt markers of toxicity, which could have contributed to a lack of a tumorigenic response.

Our data indicate that the environmentally relevant doses of BPA function in a distinctly different manner than the regulatory-based doses. While all doses of BPA significantly increased cell proliferation, it was only the regulatory-based doses of BPA that increased the rate of apoptosis. Because the environmentally relevant doses of BPA failed to increase the rate of apoptosis in the mammary gland to counter the high rate of cell proliferation, a significantly altered ratio of “cell turnover” (proliferation-to-apoptosis ratio) resulted. It was this ratio that best predicted the tumorigenic response of the erbB2-tg mouse model to each dose of BPA studied.

These findings support that concentrations of BPA that are achievable through normal dietary intake can be deleterious to women with HER2/erbB2 positive preneoplastic and neoplastic lesions in the breast.

To address the training aspects of this grant, I have attended and presented posters at six meetings, given two oral presentations, published a manuscript, and am making satisfactory progress towards graduation.

REFERENCES: none

APPENDICES: none